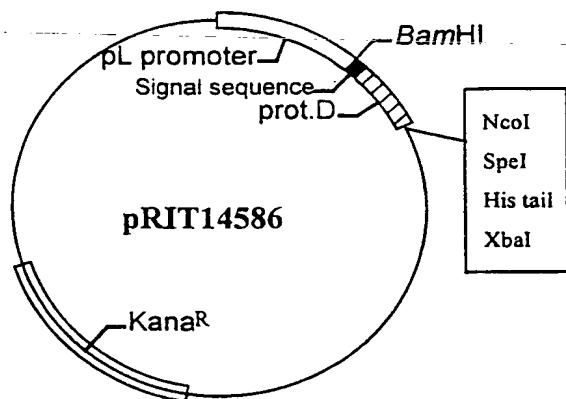


Figure 1: A/ Map of plasmid pRIT14586**B/ Coding sequence of the first 127 amino acids**

of protein D and multiple cloning site. The signal sequence is underlined.

BamHI
ATG GAT CCA AAA ACT TTA GCC CTT TCT TTA TTA GCA GCT GGC GTC CTA GCA GGT TGT AGC AGC
 Met Asp Pro Lys Thr Leu Ala Leu Ser Leu Leu Ala Ala Gly Val Leu Ala Gly Cys Ser Ser
 CAT TCA TCA AAT ATG GCG AAT ACC CAA ATG AAA TCA GAC AAA ATC ATT ATT GCT CAC CGT GGT
 His Ser Ser Asn Met Ala Asn Thr Gln Met Lys Ser Asp Lys Ile Ile Ile Ala His Arg Gly
 GCT AGC GGT TAT TTA CCA GAG CAT ACG TTA GAA TCT AAA GCA CTT GCT TTT GCA CAA CAG GCT
 Ala Ser Gly Tyr Leu Pro Glu His Thr Leu Glu Ser Lys Ala Leu Ala Phe Ala Gin Gin Ala
 GAT TAT TTA GAG CAA GAT TTA GCA ATG ACT AAG GAT GGT CGT TTA GTG GTT ATT CAC GAT CAC
 Asp Tyr Leu Glu Gln Asp Leu Ala Met Thr Lys Asp Gly Arg Leu Val Val Ile His Asp His
 TTT TTA GAT GGC TTG ACT GAT GTT GCG AAA AAA TTC CCA CAT CGT CAT CGT AAA GAT GGC CGT
 Phe Leu Asp Gly Leu Thr Asp Val Ala Lys Lys Phe Pro His Arg His Arg Lys Asp Gly Arg
 TAC TAT GTC ATC GAC TTT ACC TTA AAA GAA ATT GAA AGT TTA GAA ATG ACA GAA AAC TTT GAA
 Tyr Tyr Val Ile Asp Phe Thr Leu Lys Glu Ile Gln Ser Leu Glu Met Thr Glu Asn Phe Glu
NcoI Spel XbaI
ACC ATG GCC ACG TGT GAT CAG AGC TCA ACT AGT GGA CAC CAT CAC CAT TAA TCT AGA
 Thr Met Ala Thr Cys Asp Gin Ser Ser Thr Ser Gly His His His His His His His His His

The amino acid sequence of Figure 1 relates to Seq. ID no. 7 and the nucleic acid sequence of Figure 1 relates to Seq. ID. No. 6.

The DNA and amino acid sequences of Nef-His; Tat-His; Nef-Tat-His fusion and mutated Tat is illustrated.

Pichia-expressed constructs (plain constructs)

⇒ Nef - HIS

DNA sequence (Seq. ID. No. 8)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTGTGCTGG
CTAGAAGCACAAAGAGGAGGAGGAGGTGGGTTTCCAGTCACACCTCAGGTACCTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTGGATGGTGTACAAGCTAGTACCAAGTTGAGCCAGATAAG
GTAGAAAGAGGCCAATAAAGGAGAGAACACCAGCTTACACCCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAAGTGTAGAGTGGAGGTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGC
CACCACCATCACCATTAA

Protein sequence (Seq. ID. No. 9)

MGWKWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEVGFPVTPQVPLRPMTYKAADVLSHFLKEKGLEGLIHSQRQDILDLWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFDSRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ Tat - HIS

DNA sequence (Seq. ID. No. 10)

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAA
ACTGCTTGTACCAATTGCTATTGTAAAAAGTGTGCTTCATTGCCAAGTTGTTTC
ATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
CCTCCTCAAGGCAGTCAGACTCATCAAGTTCTATCAAAGCAACCCACCTCCCAA

TCCCGAGGGGACCCGACAGGCCCGAAGGAAACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence (Seq. ID. No. 11)

MEPVDPRLPWKHPGSQPKTACTNCYCKCCFHCQVCFITKALGISYGRKKRRQRRR
PPQGSQTHQVSLSKQPTSQSQRDPGPKETSGHHHHHH.

⇒ Nef - Tat - HIS

DNA sequence (Seq. ID. No. 12)

ATGGGTGGCAAGTGGTAAAAAGTAGTGTGGTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATAACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAACAGAACAGAGGAGGAGGAGGTGGGTTTCCAGTCACACCTCAGGTACCTTA
AGACCAATGACTTACAAGGAGCTGTAGATCTTAGCCACTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACTTCCAAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCAACACAAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGTC
AGATATCCACTGACCTTGGATGGTGCCTACAAGCTAGTACCAAGCTGTGAGCCAGATAAG
GTAGAACAGGCCAATAAAGGAGAGAACACCAGCTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCCTGAGAGAGAACAGCTGTGTTAGAGTGGAGGTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAG
CCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAATGCT
TGTACCAATTGCTATTGTTAAAAGTGTGCTTCATTGCCAAGTTGTTCATACAA
AAAGCCTTAGGCATCTCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCT
CAAGGCAGTCAGACTCATCAAGTTCTATCAAAGCAACCCACCTCCCAATCCCGA
GGGGACCCGACAGGCCGAAGGAAACTAGTGGCCACCATCACCATCACCATTAAC

Protein sequence (Seq. ID. No. 13)

^

MGGKWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAISSNTAATNAACAW
LEAQEEEVGFPTVPLRPMTYKAAVDSLHFLKEKGGLLEGLIHSQRQDILDLWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLPVPEPDKVEEANKGENTSLHPVSLH
GMDDPEREVLEWRFDSSLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTA
CTNCYCKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQR
GDPTGPKETSGHHHHHH.

E.coli-expressed constructs (fusion constructs)

⇒ LipoD-Nef-HIS

DNA sequence (Seq. ID. No. 14)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.
The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAAACTTAGCC~~TTT~~**CTT**TATTAGCAG**CTGGCGTAC**TAGCAGGTTTGT
AGCAGCCATT**CATCAA**ATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
GCTCACC**GTGGT**GCTAGCGGTTATTAC**CAAGAGC**ATACGTTAGAATCTAAAGC**ACTT**
GCTTTGCACAAACAGGCTGATTATTAGAGCAAGAT**TTAGC**AAGTGACTAAGGGATGGT
CGTTTAGTGGTTATT**CACGAT**CACTTTAGATGG**CTTGACT**GATGG**TCGAA**AAAAAA
TTCCCACAT**CGTCAT**CGAAAGATGGCCTTACTAT**GTCAT**CGACTTAC**CTTAA**AA
GAAATT**CAAAG**TTAGAAATGACAGAAAC**TTGAA**ACC**ATGGGT**GG**CAAGTGG**TCA
AAAAGTAGTGTGGTTGGATGG**C**TACT**GTAAGG**AAAGAATGAGACGAG**CTGAGCC**
GCAGCAGATGGGGTGGAGCAGC**ATCTGAGAC**CTGGAAAAACATGGAG**CAATCACA**
AGTAG**CAATAC**AGCAG**CTACCA**ATG**CTGCTTG**C**CTGG**C**TAAG**AGC**ACAAG**AGGAG
GAGGAGGTGGGTTTC**CAGTCACAC**CTCAG**GTAC**CTTAAG**ACCA**ATG**ACTTAC**AAG
GCAG**CTGTAG**AT**CTTAGCC**ACT**TTTAA**AGAAAGGGGGACTGG**AAGGG**CTAATT
CACT**CCCAAC**GAAG**ACAAG**AT**TCC**TTG**ATCTGTGG**AT**CTACCAC**AC**ACAAG**G**CTAC**
TT**CCCTGATTGG**CAG**AACTAC**AC**ACACCAGGGCC**AGGG**TCAG**AT**ATCC**ACT**GAC**CTT
GG**ATGGT**G**CTACAAG**CT**AGTAC**CA**GGTAC**CCAG**ATAAGG**TAGAAG**AGGGCC**AA**AAA**
GG**AGAGAACACCAG**CT**TGTTAC**CC**CTGTGAGC**CT**GCAT**GG**AATGG**AT**GAC**CT**GAG**
AGAGAAGT**GTTAGAGT**GG**GAGGTT**G**ACAGCCG**C**CTAGC**ATT**CAT**AC**GTGGCC**CA**CCAT**AC**CCAT**
TAA

Protein sequence of the processed lipidated ProtD-Nef-HIS protein (Seq. ID. No. 15)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQM**KSDK**IIIAHRGAS**GYLPEHT**LES**KALAF**AQ**QADYL**E**QDLAMTKD**
GRLVVI**HDHFLDGL**TDVAK**KFPHRHRKD**GRYY**VIDFTL**KE**IQSLEM**TENFET**MGK**W
SKSSVVG**WPTVR**ERM**RRAEPA**AD**GVGA**ASRD**LEKHGA**IT**SSNTA**AT**NAACAW**LE**AQE**
EEEV**GFPVTP**QVPLRPM**TYKAA**VD**L**SHFL**KEKG**GLE**GLIHSQRRQD**I**LDLW**I**YHTQG**
YFPDW**QNYTPGPGVRY**PLTFG**WCYK**LVP**VEPDK**V**EEANK**G**ENT**SLL**HPV**SL**HGMDDP**
EREV**LEWRFDSRLAFHH**AREL**HPEYFK**NC**TSGH**HHHHH.

⇒ LipoD-Nef-Tat-HISDNA sequence (Seq. ID. No. 16)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

```

ATGGATCCAAAAACTTAGCCTTCTTATTAGCAGCTGGCGTACTAGCAGGTTGT
AGCAGCCATTCATCAAATATGGCGAATACCCAATGAAATCAGACAAATCATTTATT
GCTCACCGTGGTGCTAGCGGTTATTACCAGAGCATACGTTAGAATCTAAAGCACTT
GCGTTTGACAACAGGCTGATTATTAGAGCAAGATTAGCAATGACTAAGGATGGT
CGTTTAGTGGTTATTCACGATCACTTTAGATGGCTTGACTGATGTTGCGAAAAAAA
TTCCCACATCGTCATCGTAAAGATGGCCTTACTATGTCATCGACTTTACCTTAAAAA
GAAATTCAAAGTTAGAAATGACAGAAAACTTGAAACCATGGGTGGCAGTGGTCA
AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAACATGGAGCAATCACA
AGTAGCAATACAGCAGCTACCAATGCTGCTTGCTGGCTAGAAGCACAAGAGGAG
GAGGAGGTGGGTTTCCAGTCACACCTCAGGTACCTTAAGACCAATGACTTACAAG
GCAGCTGTAGATCTTAGCCACTTTAAAAGAAAAGGGGGACTGGAAGGGCTAATT
CACTCCCAACGAAGACAAGATATCCTGATCTGTGGATCTACCACACACAAGGCTAC
TTCCCTGATTGGCAGAACTACACACCAGGCCAGGGTCAGATATCCACTGACCTT
GGATGGTGCTACAAGCTAGTACCAGGCCAGATAAGGTAGAAGAGGCCAATAAA
GGAGAGAACACCAGCTTGTACACCCTGTGAGCCTGCATGGAATGGATGACCTGAG
AGAGAAGTGTAGAGTGGAGGTTGACAGCCGCCTAGCATTCATCACGTGGCCCGA
GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTA
GAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACTGCTTGTACCAATTGCTATTGT
AAAAAGTGTGCTTCATTGCCAAGTTGTTCATAACAAAAGCCTAGGCATCTCC
TATGGCAGGAAGAACGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCAT
CAAGTTCTCTATCAAAGCAACCCACCTCCAATCCGAGGGGACCCGACAGGCCG
AAGGAAACTAGTGGCCACCATCACCATCACCATTAA

```

Protein sequence of the processed lipidated ProtD-NEF-TAT-HIS protein (Seq. ID. No. 17)

(Amino-acids corresponding to Prot D fusion partner are in bold)

```

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFAQQADYLEQDLAMTKD
GRLVVIHDHFLDGLTDVAKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGGKW
SKSSVVGWPTVRERMRRAEPADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
EEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRQDILDLWIYHTQG
YFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLHPVSLHGMDDP
EREVLEWRFDSRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCY
CKKCCFHCQVCFITKALGISYGRKKRQRRPPQGSQTHQVSLSKQPTSQSRGDPTG
PKETSGHHHHHH.

```

\Rightarrow ProtD-Nef-HIS

DNA sequence (Seq. ID. No. 18)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACCGTGGTCTAGCGGTTATTACCAAGAGCATACGTTAGAATCT
AAAGCACTTGCCTTGACAAACAGGCTGATTATTAGAGCAAGATTAGCAATGACT
AAGGATGGTCGTTAGTGGTTATTACGATCACTTTTAGATGGCTTACTATGTCATCGACTTT
GCGAAAAAAATTCCCACATCGTACATCGTAAAGATGCCGTTACTATGTCATCGACTTT
ACCTTAAAAGAAATTCAAAGTTAGAAATGACAGAAAACCTTGAAACCATTGGGTGGC
AAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTGAGACCTGGAAAAACATGGG
GCAATACAAGTAGCAATACAGCAGCTACCAATGCTGTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGTTCCAGTCACACCTCAGGTACCTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTAAAAGAAAAGGGGGACTGGAA
GGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGTCAGATATCCA
CTGACCTTGGATGGTGCCTACAAGCTAGTACCAAGCTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTACACCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTAGAGTGGAGGTTGACAGCCGCCTAGCATTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCAC
CATCACCATTAA

Protein sequence (Seq. ID. No. 19)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQQADYL
EQDLAMTKDGRLLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLK
EIQSLEMTENFETMGGKWSKSSVVGWPTVRERMRRRAEPAAADGVGAASRDL
EKHGAITSSNTAATNAACAWLEAQEEEVGFPVTPQVPLRPMTYKAADVLSH
FLKEKGGLLEGLIHSQRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGW
CYKLVPVEPDKVEEANKGENTSLHPVSLHGMDDPEREVLEWRFDSRLAFH
HVARELHPEYFKNCTSGHHHHHH.

⇒ ProtD-Nef-Tat-HIS

DNA sequence (Seq. ID. No. 20)

7/17

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
 ATCATTATTGCTCACCGTGGTAGCGGTTATTTACAGAGCATACTGTTAGAATCT
 AAAGCACTTGCCTTGCACAACAGGCTGATTATTTAGAGCAAGATTAGCAATGACT
 AAGGATGGTCGTTAGGGTTATTACGATCACTTTAGATGGCTGACTGATGTT
 GCGAAAAAAATTCCCACATCGTCATCGTAAAGATGGCCGTACTATGTCATCGACTTT
 ACCTTAAAAGAAATTCAAAGTTAGAAATGACAGAAAACCTTGAAACCATGGGTGGC
 AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
 GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
 GCAATCACAAGTAGCAATAACAGCAGCTACCAATGCTGCTGTGCCTGGCTAGAAGCA
 CAAGAGGAGGAGGAGGTGGGTTTCCAGTCACACCTCAGGTACCTTAAGACCAATG
 ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTAAAAGAAAAGGGGGACTGGAA
 GGGCTAATTCACTCCCAACGAAGACAAGATATCCTGATCTGTGGATCTACCACACA
 CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGTCAGATATCCA
 CTGACCTTGATGGTCTACAAGCTAGTACCAAGCTGAGCCAGATAAGGTAGAAGAG
 GCCAATAAAGGAGAGAACACCAGCTGTTACACCCCTGTGAGCCTGCATGGAATGGAT
 GACCCTGAGAGAGAAGTGTAGAGTGGAGGTTGACAGCCGCTAGCATTTCATCAC
 GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGAT
 CCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAAGTGTGTTACCAAT
 TGCTATTGTAAAAGTGTGCTTCATTGCCAAGTTGTTCATAAACAAAAGCCTTA
 GGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGT
 CAGACTCATCAAGTTCTATCAAAGCAACCCACCTCCAATCCGAGGGGACCCG
 ACAGGCCGAAGGAAACTAGTGGCCACCATCACCACATCACCATTAA

Protein sequence (Seq. ID. No. 21)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHS SNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQQADYLEQDLAMT
 KDGRIVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTEFNFTMGG
 KWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEA
 QEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLLEGLIHSQRQDILDLWIYHT
 QGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSSLHPVSLHGMD
 DPEREVLEWRFDSRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTN
 CYCKKCCFHCQVCFITKALGISYGRKKRQRRLPPQGSQTHQVSLSKQPTSQRGDP
 TGPKETSGHHHHHH .

⇒ Tat-MUTANT-HIS

DNA sequence (Seq. ID. No. 22)

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATC	40
CAGGAAGTCAGCCTAAAAGTCTTGTACCAATTGCTATTG	80
TAAAAAGTGTGCTTCATTCCAAGTTGTTCATACAA	120
GCTGCCTTAGGCATCTCCTATGGCAGGAAGAACGGAGAC	160
AGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGT	200
TTCTCTATCAAAGCAACCCACCTCCAATCCAAAGGGAG	240
CCGACAGGCCGAAGGAAACTAGTGGCCACCATCACCATC	280
ACCATTAA	288

Protein sequence(Seq. ID. No. 23)

Mutated amino-acids in Tat sequences are in bold.

MEPVDPRLPWPKHPGSQPKTACTNCYCKKCCFHCQVCFIT	40
AALG ISYGRKKRRQRRPPQGSQTHQVSLSKQPTSQSKE	80
PTGPKETSGHHHHHH.	95

⇒*Nef-Tat-Mutant-HIS*

DNA sequence(Seq. ID. No. 24)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGC	40
CTACTGTAAGGAAAGAACGAGACGAGCTGAGCCAGCAGC	80
AGATGGGGTGGAGCAGCATCTCGAGACCTGGAAAAACAT	120
GGAGCAATCACAAGTAGCAATAACAGCAGCTACCAATGCTG	160
CTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGG	200
TTTCCAGTCACACCTCAGGTACCTTAAGACCAATGACT	240
TACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAAGAAA	280
AGGGGGGACTGGAAGGGCTAATTCACTCCAACGAAGACA	320
AGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC	360
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGTCA	400
GATATCCACTGACCTTGGATGGTGTACAAGCTAGTACC	440
AGTTGAGCCAGATAAGGTAGAAGAGGCCATAAAGGAGAG	480
AACACCAGCTTGTACACCCTGTGAGCCTGCATGGAATGG	520
ATGACCCCTGAGAGAGAAGTGTAGAGTGGAGGTTGACAG	560
CCGCCTAGCATTTCATCACGTGGCCGAGAGCTGCATCCG	600
GAGTACTTCAAGAACTGCACTAGTGTAGCCAGTAGATCCTA	640
GAATAGAGCCCTGGAAGCATTCCAGGAAGTCAGCCTAAAC	680
TGCTTGTACCAATTGCTATTGTAAGGTGTGCTTTCAT	720
TGCCAAGTTGTTCATAACAGCTGCCTTAGGCATCTCCT	760
ATGGCAGGAAGAACGGAGACAGCGACGAAGACCTCCTCA	800
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ACCTCCCAATCCAAAGGGAGCCGACAGGCCGAAGGAAA	880
CTAGTGGCCACCATCACCACCAATTAA	909

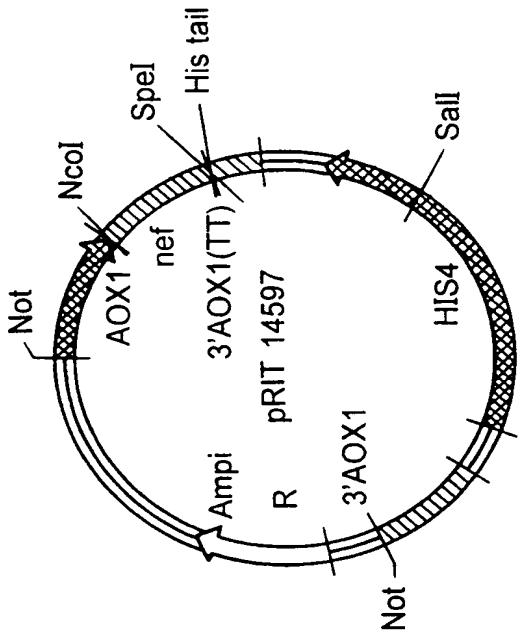
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Protein sequence (Seq. ID. No. 25)

Mutated amino-acids in Tat sequence are in bold.

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKH	40
GAITSSNTAATNAACAWLEAQEEEVGFPVTPQVPLRPMT	80
YKAAVDLSHFLKEKGGLIHSQRRQDILDLWIYHTQGY	120
FPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGE	160
NTSLLHPVSLHGMDDPEREVLEWRFDSRLAFHHVARELHP	200
EYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFH	240
CQVCFITAALGISYGRKKRRQRRPPQGSQTHQVSLSKQP	280
TSQS K GEP T GP K ETSGHHHHHH.	302

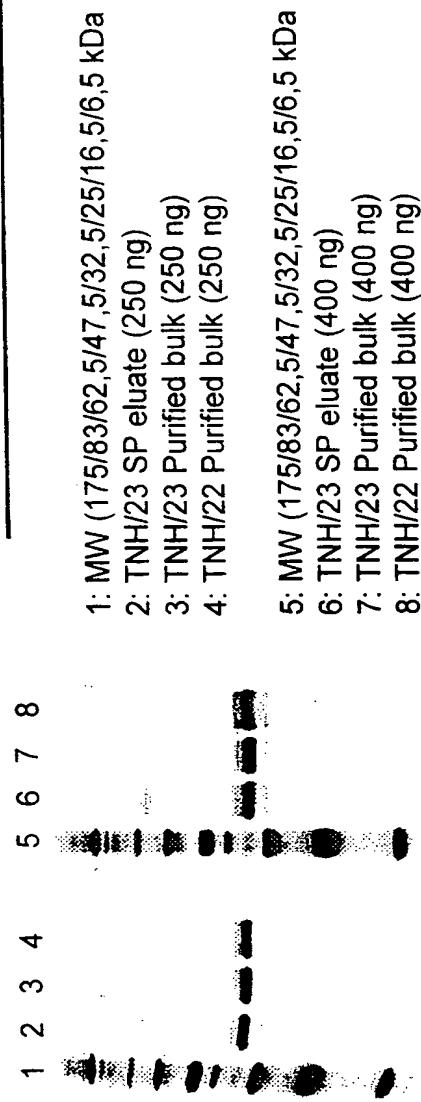
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Fig . 3 Map of pRIT14597 integrative vector

MCS POLYLINKER: *nef* gene inserted between *NcoI* and *SpeI* sites.

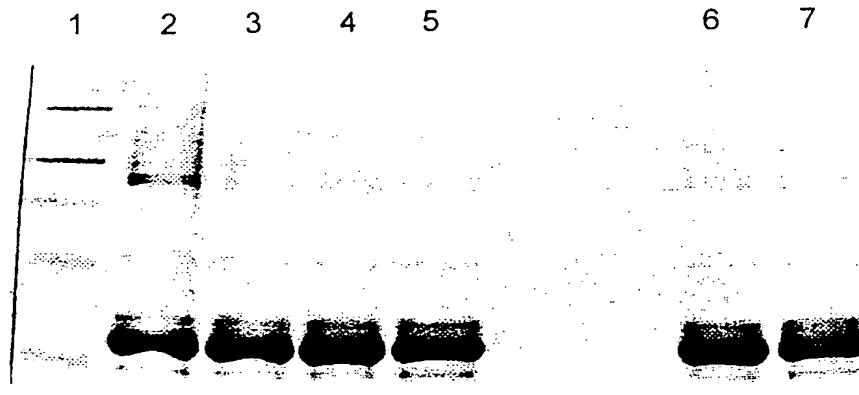
<i>Acu II</i>	<i>Nco I</i>	<i>Spe I</i>	<i>Eco RI</i>
TTCGAAACC	ATGGCCGGACTAGT	GGC.CAC.CAT.CAC.CAT.TAA	CGGAATTTC
		Thr . Ser . Gly . His . His . His . His . His	

The amino acid sequence of Figure 3 relates to Seq. ID no. 27 and the nucleic acid sequence of Figure 3 relates to Seq. ID. No.26.

Fig. 4 SDS-PAGE: Nef-Tat-his fusion proteinDaiichi Silver Staining

1 2 3 4

Blot Nef-Tat (LAS 97340)Blot Tat2

Fig . 5 SDS-PAGE: Nef-Tat-his fusion proteinCoomassie blue G250

- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa)
- 2: TNH/23 SP eluate (4 µg)
- 3: TNH/23 Superdex200 eluate (4 µg)
- 4: TNH/23 Purified bulk (4 µg)
- 5: TNH/22 Purified bulk (4 µg)
- 6: TNH/23 Purified bulk (4 µg) / non reducing conditions
- 7: TNH/22 Purified bulk (4 µg) / non reducing conditions

Fig. 6A Tat-specific antibody titers and isotypes

group	immunization	midpoint titers			ratio IgG1/IgG2a	
		Ig	IgG1	IgG2a		
1	oxydized Tat	3533557	135538	98771	98763	1,372
2	reduced Tat	252275	72087	76273	72014	0,945
3	oxydized Nef-Tat	246466	179616	60835	53563	2,953
4	reduced Nef-Tat	91726	73767	30948	20679	2,384
5	adjuvant only	<4000	<4000	<4000	<4000	

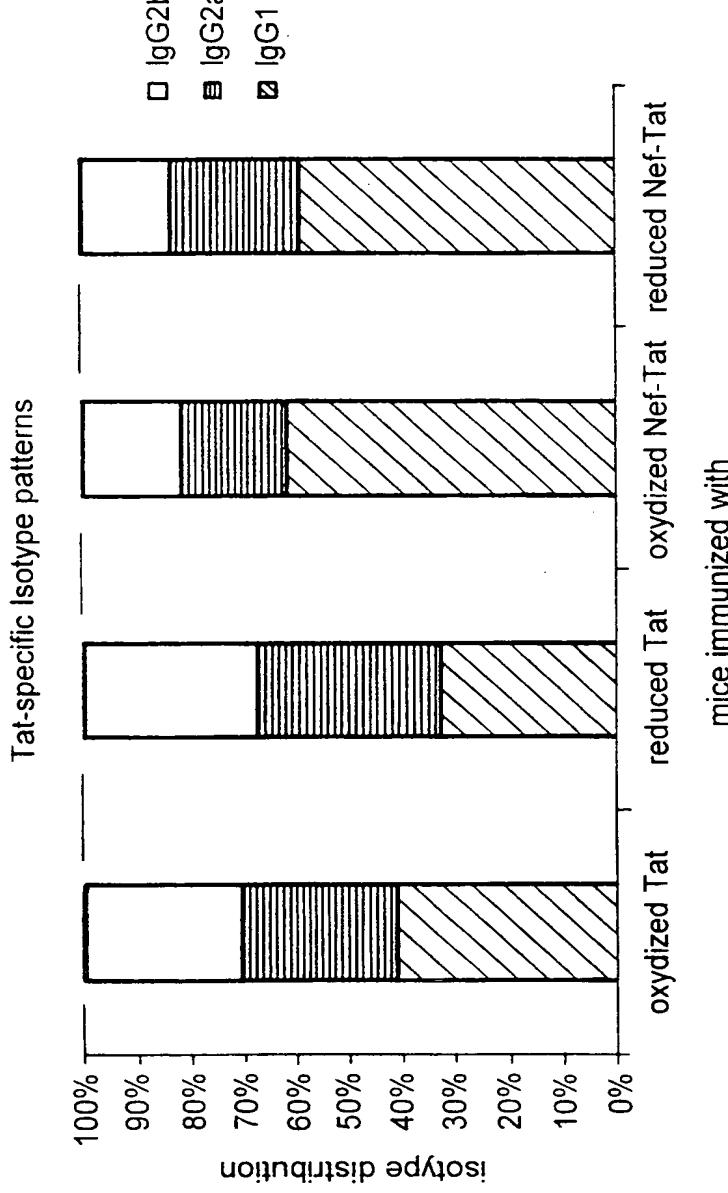


Fig. 6B Tat-specific antibody titers and isotypes

group	immunization	midpoint titers				ratio IgG1/IgG2a
		Ig	IgG1	IgG2a	IgG2b	
1	reduced Tat	212799	123242	62697	55763	1,966
2	reduced Nef-Tat	75676	84046	18449	11692	4,556
3	adjuvant only	<4000	<4000	<4000	<4000	

Tat-specific Isotype patterns

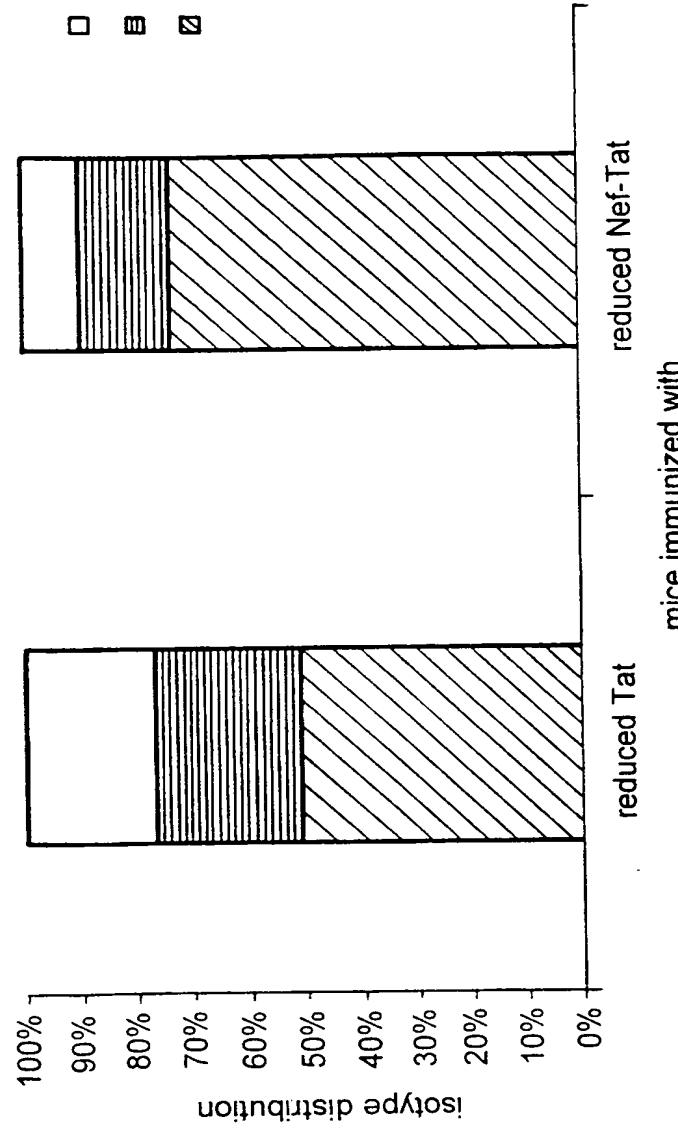


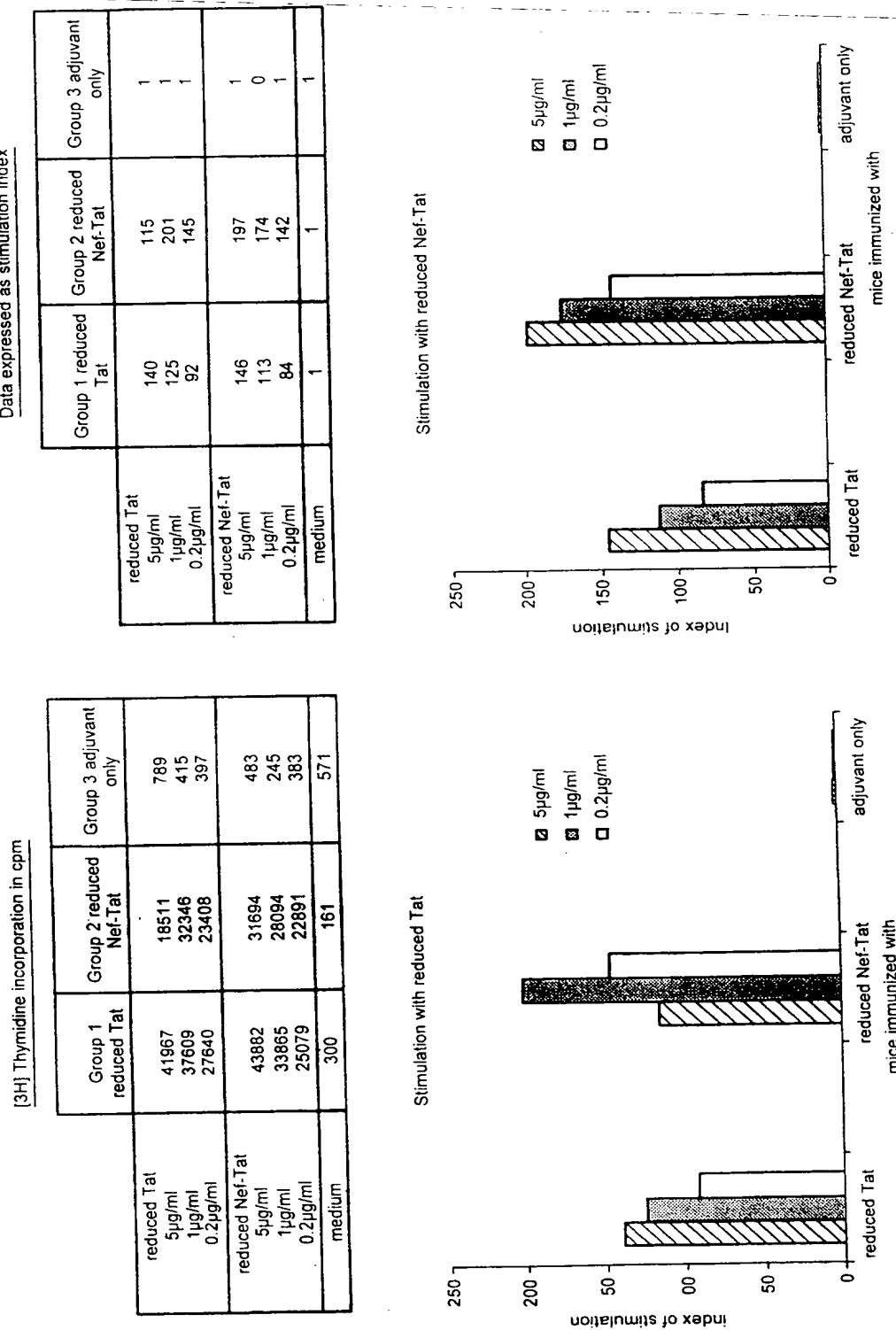
Fig. 7 Antigen-specific lymphoproliferative response of pooled lymph node cells

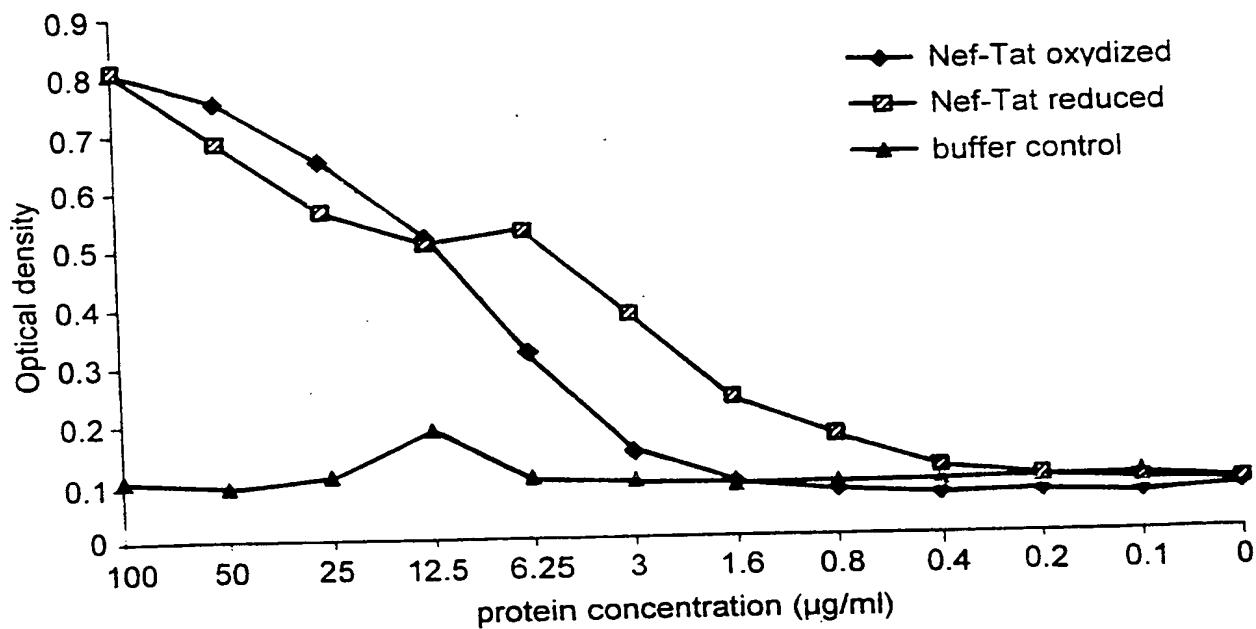
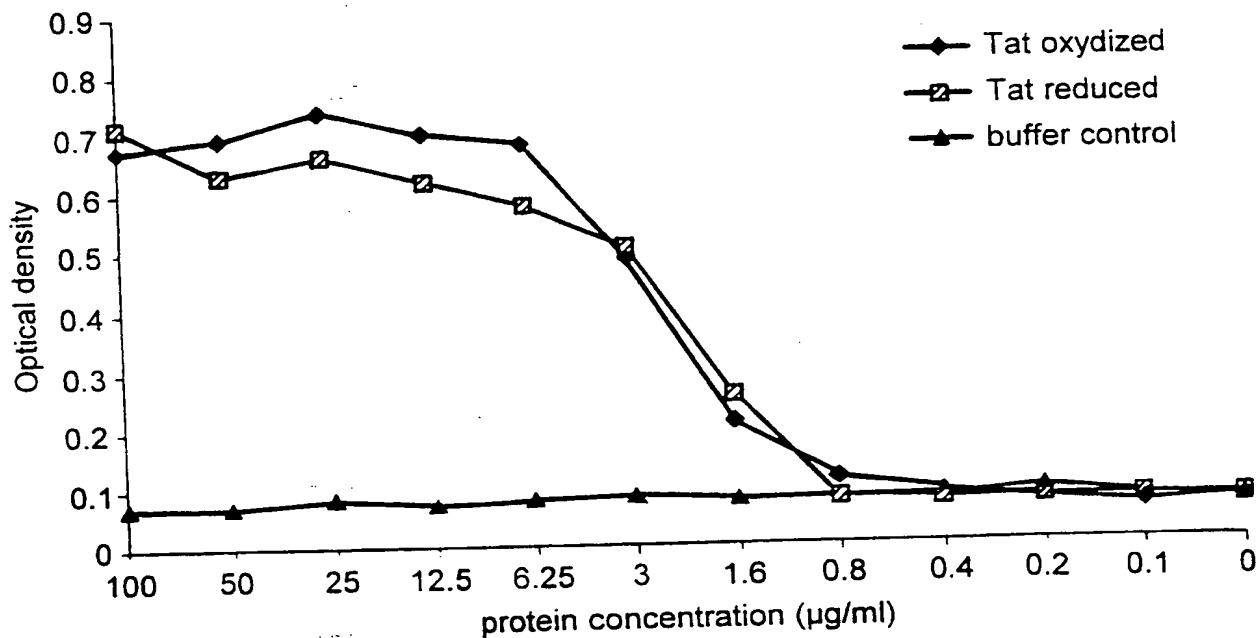
Fig. 8 Cell binding assay

Fig. 9 Inhibition of cell growth